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Impact of Donor-Recipient Major ABO Mismatch on Allogeneic Transplantation Outcome According to Stem Cell Source

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Major ABO incompatibility between donor and recipient is not considered a barrier to successful allogeneic hematopoietic stem cell transplantation (HSCT), even if it can be associated with several immunohematologic complications. Nevertheless, conflicting data still exist as to its influence on graft-versus-host disease (GVHD) incidence, relapse rate, and survival. To further investigate the relevance of ABO major mismatch on transplantation outcome, we retrospectively analyzed results from 414 patients with major or major/minor ABO-mismatched bone marrow (BM), peripheral blood (PB), and cord blood (CB) allogeneic HSCT. Transplantation outcome was assessed by comparison with results from a 395-patient ABO-compatible population with similar characteristics. Median time to red cell transfusion independence was significantly longer in ABO-incompatible BM recipients (median time, 63 days vs 41 days; $P = .001$), with faster disappearance of antidonor IgM hemagglutinins in unrelated recipients (median time, 36 days vs 44 days; $P = .03$) and in patients with grade ≥II acute GVHD (aGVHD) (median time, 35 days vs 59 days ; $P = .001$). In PB stem cell (PBSC) and CB transplantation, erythroid reconstitution was not significantly delayed, regardless of donor type or presence of aGVHD. A slight correlation between ABO incompatibility and GVHD incidence was found in PBSC recipients when considering grade ≥II aGVHD incidence (63% in ABO-matched HSCT vs 83% in ABO-mismatched HSCT; $P = .055$), but this was not confirmed in multivariate analysis. In patients with acute leukemia, multivariate analysis revealed an association between major ABO mismatch and decreased relapse rate with borderline statistical significance (hazard ratio, 0.65; $P = .04$). Major ABO incompatibility mainly, if not exclusively, affects red blood cell engraftment after BM transplantation. Somewhat surprisingly, the graft-versus-plasma cell effect seems to be confined to this stem cell source.

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KEY WORDS: Acute graft-versus-host disease, Bone marrow, ABO incompatibility, Hematopoietic recovery, Transfusion needs

INTRODUCTION

In contrast to solid organ transplantation, allogeneic hematopoietic stem cell transplantation (HSCT) can be performed across the ABO blood group barrier [1]. ABO incompatibility between donor and recipient occurs in 30%-40% of patients undergoing HSCT, because ABO blood groups are inherited independently from human leukocyte antigens [2].

Three groups of ABO mismatch can be distinguished in HSCT: minor, major, and bidirectional ABO incompatibility. Minor ABO incompatibility (eg, from an type-O donor to a type-A, -B, or -AB recipient) is characterized by the ability of donor B lymphocytes to produce antirecipient isoagglutinins. In contrast, major ABO-incompatible HSCT (eg, from a type-A, -AB, or -B donor to an type-O recipient) is characterized by the presence of preformed antidonor isoagglutinins. In bidirectional ABO

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incompatibility (eg, type-A donor to a type-B recipient), a combination of both major and minor ABO blood group barriers must be overcome.

Although ABO incompatibility between donor and recipient does not represent a barrier to successful HSCT, it is well established that major ABO incompatibility can lead to prolonged destruction of donor-derived erythrocytes, with pure red blood cell (RBC) aplasia and prolonged transfusion requirements [3], despite such techniques as plasma exchange and RBC depletion of the donor marrow [4,5]. Similarly, minor ABO incompatibility can result in an increased risk of delayed immune hemolysis [6,7], which occurs in approximately 10%-15% of cases [1].

Mielcarek et al. [8] reported that the degree of genetic disparity between donor and recipient, especially in unrelated graft recipients, and graft-versus-host disease (GVHD) can affect the rate of disappearance of antidonor isohemagglutinins, suggesting a graft-versus–plasma cell effect. More recently, Kimura et al. [9] reported specific effects of major and minor ABO incompatibility on transplantation-related mortality and incidence of acute GVHD (aGVHD) in recipients of bone marrow transplants (BMTs) from unrelated donors. However, conflicting data still exist about the influence of major ABO incompatibility on GVHD incidence [8,10], hematologic recovery [11], relapse rate [12,13], and survival [14-16], especially with the use of alternative donor sources, such as peripheral blood (PB) and cord blood (CB).

Allogeneic PB stem cell transplantation (PBSCT) has been increasingly preferred over BMT because of its more rapid hematologic recovery and potent graft-versus-tumor effect in hematologic malignancies [17,18]. However, to date, only a few studies have addressed the impact of major ABO mismatch on GVHD incidence and survival in PBSCT.

The aims of this single-center study were to (1) validate the impact of major ABO mismatch and genetic disparity on erythroid reconstitution in allogeneic BMT; (2) investigate the role of major ABO incompatibility on hematologic recovery, GVHD incidence, relapse rate, and overall survival (OS) in PBSCT; and (3) analyze these 4 parameters in CB transplantation (CBT).

MATERIALS AND METHODS

Patient Characteristics

The study included a total of 414 patients who underwent HSCT at Saint Louis University Hospital, Paris, between 1978 and 2005. Median follow-up was 76 months. Of the 414 patients, 226 underwent BMT, 138 underwent PBSCT, and 49 underwent CBT.

Among the ABO-mismatch population, patients received a major (n = 337; 81%) or major/minor (n = 77; 19%) ABO-mismatched allogeneic transplant from a matched related donor (MRD; n = 136; 33%) or a matched unrelated donor (MUD; n = 278; 67%). Myeloablative (MA) conditioning regimens included fractioned total body irradiation (TBI) + cyclophosphamide (n = 166; 40%) and busulfan + cyclophosphamide (n = 168; 41%). Nonmyeloablative (NMA) regimens were all fludarabine-based, either with (n = 20; 5%) or without (n = 60; 14%) 2 Gy TBI. HSCT was performed for both malignant (n = 296; 71%) and nonmalignant (n = 118; 29%) hematologic diseases, and GVHD prophylaxis was based mainly on standard regimen with cyclosporine A (CsA) + methotrexate (n = 312; 75%) after MA conditioning and with cyclosporine A (CsA) + mycophenolate mofetil (MMF; n = 73 [18%]) after NMA conditioning. Details regarding indications for HSCT, GVHD prophylaxis, GVHD incidence, and other patient and transplant characteristics are given in Table 1.

Transplantation outcomes were compared with those from a 395-patient ABO-compatible population with similar characteristics with respect to period of transplantation, stem cell source, conditioning regimen, GVHD prophylaxis, and donor type (Table 2).

Hemagglutinin Titer Monitoring and Transfusion Policy

As described previously [8,19], posttransplantation anti-A and anti-B isohemagglutinin IgG and IgM titers were followed weekly from day +4 post-HSCT for each patient. For patients with an anti-A and/or anti-B titer >1:128 during pretransplantation assessment, IgG and IgM were evaluated twice weekly after transplantation until achievement of titers below 1:16, then weekly until their complete disappearance. Hemagglutinin titer quantification was followed until it was undetectable for 2 consecutive weeks, except in patients with persistent RBC transfusion requirements.

All transfused packed RBCs and platelets were separated from plasma and irradiated at a dose of 30 Gy to prevent the risk of acute transfusion-induced GVHD. Whatever the period of transplant was, all patients received recipient- or type-O RBCs as long as isoagglutinins directed against the donor blood group remained measurable. At that point, RBC transfusions were switched to donor type.

Statistical Analysis

OS within ABO compatibility groups for all stem cell source and donor type was estimated by the Kaplan-Meier method [20]. For survival analysis, follow-up time was censored at the date of last contact for surviving patients, and the log-rank test was used to test the significance of differences [21], adjusting for potential confounding variables (age, disease status,

Table 1. ABO-Mismatched Transplantations: Patients and Transplant Characteristics

Parameter	MRD	MUD	*P*
Total, n (%)	136 (33)	278 (67)	NA
Sex, % male	58	54	.68
Age, years, median (range)	26.2 (3-55)	24.3 (0.6-66)	.75
Hematologic diagnosis, n (%)			
AML	30 (22)	57 (21)	.65
ALL	24 (18)	52 (19)	.76
MDS	9 (7)	18 (6)	.60
CML	31 (23)	53 (19)	.32
SAA	14 (10)	31 (11)	.40
PNH	3 (2)	3 (1)	.86
Hodgkin lymphoma	2 (1)	4 (1)	.90
NHL	5 (4)	11 (4)	.76
Fanconi anemia	11 (8)	26 (9)	.67
Sickle cell anemia/thalassemia	3 (2)	7 (3)	.80
Miscellaneous	4 (3)	16 (6)	.26
Stem cell source, n (%)			
BM	72 (53)	154 (55)	.43
PBSC	56 (41)	82 (29)	**.05**
CB (simple)	8 (6)	34 (12)	.16
CB (double)	0 (0)	7 (3)	.65
BM + CB	0 (0)	1 (1)	.90
Conditioning regimen, n (%)			
TBI/Cy	52 (38)	114 (41)	.35
Bu/Cy	58 (43)	110 (40)	.42
Other TBI-containing regimens	7 (5)	13 (4)	.60
Other non–TBI-containing regimens	19 (14)	41 (15)	.45
Myeloablative	110 (81)	226 (81)	.90
Nonmyeloablative	26 (19)	52 (19)	.85
GVHD prophylaxis, n (%)			
CsA-MTX	100 (74)	212 (76)	.65
CsA alone	6 (4)	10 (4)	.80
CsA-MMF	23 (17)	50 (18)	.65
MMF-corticosteroids	3 (2)	4 (1)	.87
MTX alone	4 (3)	2 (1)	.80
aGVHD, n (%)			
Grade 0-I	66 (49)	44 (16)	**.01**
Grade II-IV	70 (51)	234 (84)	**.01**
ABO incompatibility, n (%)			
Major mismatch	108 (79)	229 (82)	.26
Major/minor mismatch	28 (21)	49 (18)	.40
Hemagglutinin type, n (%)			
Anti-A	89 (65)	185 (67)	.41
Anti-B	44 (32)	86 (31)	.50
Anti-A + anti-B	3 (2)	7 (2)	.90

AML indicates acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; MDS, myelodysplastic syndrome; CML, chronic myelogenous leukemia; SAA, severe aplastic anemia; PNH, paroxysmal nocturnal hemoglobinuria; NHL, non-Hodgkin lymphoma; Cy, cyclophosphamide; Bu, busulfan; CsA, cyclosporine A; MTX, methotrexate.

Table 2. Characteristics of ABO-Matched and ABO-Mismatched Populations

Parameter	ABO Mismatch	ABO Match	*P*
Total, n (%)	414 (51)	395 (49)	NA
Sex, % male	55	54	.42
Age, years, median (range)	25.3 (0.6-66)	28.6 (3-60)	.35
Hematologic diagnosis, n (%)			
AML	87 (12)	83 (10)	.39
ALL	76 (9)	74 (9)	.46
MDS	27 (3)	27 (3)	.50
CML	84 (10)	84 (10)	.30
SAA	45 (6)	45 (6)	.42
PNH	6 (1)	5 (1)	.41
Hodgkin	6 (1)	8 (1)	.45
NHL	16 (2)	14 (2)	.42
Fanconi anemia	37 (5)	34 (4)	.50
Sickle cell anemia/thalassemia	10 (1)	10 (1)	.41
Miscellaneous	20 (2)	11 (1)	.21
Stem cell source, n(%)			
BM	226 (27)	213 (26)	.25
PBSC	138 (17)	135 (17)	.37
CB (simple)	42 (5)	41 (5)	.18
CB (double)	7 (1)	6 (1)	.30
BM + CB	1 (1)	0 (0)	.45
Conditioning regimen, n(%)			
TBI/Cy	166 (21)	163 (20)	.45
Bu/Cy	168 (20)	154 (19)	.29
Other TBI-containing regimens	20 (2)	20 (2)	.50
Other non–TBI-containing regimens	60 (7)	58 (7)	.35
Myeloablative	336 (41)	321 (40)	.32
Nonmyeloablative	78 (10)	74 (9)	.41
GVHD prophylaxis, n (%)			
CsA-MTX	312 (38)	302 (37)	.28
CsA alone	16 (2)	10 (1)	.25
CsA-MMF	73 (9)	72 (9)	.30
MMF-corticosteroids	7 (1)	7 (1)	.43
MTX alone	6 (1)	4 (1)	.26
Donor type			
MRD	136 (17)	146 (18)	.28
MUD	278 (34)	249 (32)	.25
Period of transplantation			
1978-1985	32 (4)	28 (3)	.29
1985-1995	141 (17)	140 (17)	.42
1995-2005	241 (30)	227 (28)	.38
aGVHD, n(%)			
Grade 0-I	110 (14)	144 (17)	.16
Grade II-IV	304 (38)	251 (31)	**.06**

AML indicates acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; MDS, myelodysplastic syndrome; CML, chronic myelogenous leukemia; SAA, severe aplastic anemia; PNH, paroxysmal nocturnal hemoglobinuria; NHL, non-Hodgkin's lymphoma; Cy, cyclophosphamide; Bu, busulfan; CsA, cyclosporine A; MTX, methotrexate; MRD, matched related donor; MUD, matched unrelated donor; BM, bone marrow; CB, cord blood.

HLA match, GVHD prophylaxis and incidence, sex mismatch, and year of transplantation). Incidences of aGVHD, relapse rate, and attainment of hemagglutinin titer endpoint were calculated using cumulative incidence estimates [21].

Hematologic endpoints were defined as follows:

- Engraftment: sustained absolute neutrophil count (ANC) $\geq 0.5\ 10^9$/L for at least 5 days with no use of granulocyte colony-stimulating factor
- RBC transfusion independence: absence of transfusion needs for at least 7 days with sustained hemoglobin level ≥ 8 g/dL
- Platelet recovery: platelet count $\geq 20\ 10^9$/L with no transfusion needs for at least 72 hours

Univariate analysis was performed using χ^2 and Mann-Whitney *U* tests, whereas multivariate analysis was done using Cox proportional hazard regression models [22], with assessment of age, disease status at time of transplantation, donor type, stem cell source, and ABO major mismatch on 3-year overall survival, incidence of aGVHD and chronic GVHD (cGVHD), and relapse rate. All reported *P* values are 2-sided, and $P < .05$ is considered to indicate statistical significance.

RESULTS

Impact of ABO Major Incompatibility on Erythroid Reconstitution in BMT Recipients

To validate the relevance of our 2 populations (ABO-mismatched and control ABO-matched), we first selected patients undergoing BMT and determined the impact of ABO incompatibility on erythroid reconstitution based on RBC transfusion requirements. As shown in Table 2, the 2 populations appeared to have similar characteristics, especially in terms of stem cell source, conditioning regimen, donor type, period of transplantation, and GVHD incidence ($P > .05$ for each parameter).

In ABO-incompatible marrow recipients, median time to RBC transfusion independence was significantly longer (median time, 63 days vs 41 days; $P = .001$) (Figure 1). This difference appears to be independent of the amount of cells infused during transplantation, with a median number of $CD34^+$ cells of 2.4 10^6/kg in ABO-mismatched patients and 2.6 10^6/kg in control ABO-matched patients ($P = .28$).

Impact of Donor Type on Transfusion Needs and Antidonor Hemagglutinin Disappearance Kinetics in the ABO-Mismatched Population

To confirm the role of donor type in erythroid recovery after BMT, we evaluated RBC transfusion needs after MRD and MUD HSCT in both ABO-matched and ABO–major mismatched patients. In the MRD population, the mean number of RBC packs transfused was significantly greater in the ABO-mismatched population than in the ABO-matched population (13 vs 6; $P = .01$), but this difference did not reach statistical significance in the MUD population (12 vs 14; $P = .22$). These results are given in Table 3.

We then evaluated the role of genetic disparity on erythroid reconstitution in the same population; 154 patients in the ABO-major mismatched group received a transplant from a MUD, compared with 72 from an MRD. For the 2 groups, we determined the number of days posttransplantation required to reach undetectable antidonor IgM hemagglutinins. The disappearance of antidonor IgM hemagglutinins was significantly faster in MUD recipients (median time, 36 days vs 44 days; $P = .03$) (Figure 2).

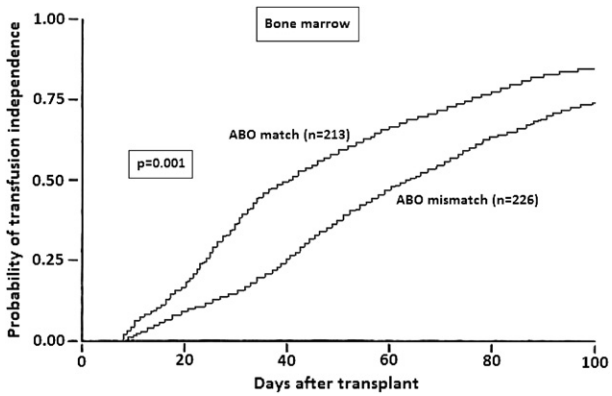

Figure 1. Probability of RBC transfusion independence according to time after transplantation in ABO-matched and major ABO-mismatched BMT recipients.

We also analyzed the correlation between the degree of HLA matching and RBC engraftment in this MUD population. Among the 154 patients receiving ABO-major mismatched BM from an MUD donor, 35 were antigen-matched and 119 were allele-matched. Among the 119 patients with allele-matched BMT, the majority (n = 87; 73%) received a 10/10 matched transplant, whereas 20 (17%) received a 9/10 matched transplant and 12 (10%) received a 8/10 matched transplant. When considering the delay after transplantation in reaching undetectable antidonor IgM hemagglutinins in those subgroups, we found a trend of shorter time for antigen-matched transplants compared with allele-matched transplants, but the difference did not attain statistical significance (32 days vs 36 days; $P = .07$). Comparing the 3 different populations according to degree of HLA allele matching (ie, 10/10, 9/10, and 8/10) revealed no significant difference, because of the small size of the corresponding subgroups (data not shown).

We investigated the effect of antithymocyte globulin (ATG) administration as part of the conditioning regimen. Among the 154 patients in the MUD group, 102 (66%) received ATG, and 52 (34%) did not. The median time to disappearance of antidonor IgM hemagglutinins was not significantly influenced by the use of ATG (34 days vs 37 days; $P = .16$).

Impact of Allogeneic Effect on Hemagglutinin Titer Clearance

After confirming the impact of donor type on the kinetic of antidonor IgM disappearance and RBC transfusion requirements, we verified the role of the allogeneic effect on the speed of clearance of hemagglutinin titers in the MUD group. For that purpose, in both the MRD and MUD groups, we compared the median time to reach undetectable antidonor IgM according to aGVHD occurrence. In the MRD group, this time was significantly shorter in patients with grade II-IV aGVHD than in those with grade 0-I aGVHD (57 vs 78 days; $P = .001$; relative risk [RR] = 1.92). In the MUD group, no significant difference was seen between these 2 groups (46 days vs 59 days; $P = .06$; RR = 1.21).

We also investigated the impact of grade II-IV aGVHD on the kinetics of transfusion independence (Figures 3 and 4). In the MRD group, the median time to RBC transfusion independence was

Table 3. Median Number of RBC Packs Transfused among ABO-Mismatched BM Recipients with MRDs and MUDs

Stem Cell Source	Donor Type	ABO Compatibility	n	RBC Packs Transfused, Median (Range)	Univariate RR	P value	95% CI
BM	MRD	ABO match	78	6 (0-26)	1.0	NA	NA
		ABO mismatch	72	13 (2-92)	**1.86**	**0.01**	1.54-2.10
	MUD	ABO match	135	14 (0-44)	1.0	NA	NA
		ABO mismatch	154	12 (4-32)	0.95	0.22	0.80-1.05

BM indicates bone marrow; MRD, matched related donor; MUD, matched unrelated donor.

significantly shorter in patients with grade II-IV aGVHD compared with those with grade 0-I aGVHD (42 days vs 80 days; $P = .001$). Of the MRD recipients with grade II-IV aGVHD (n = 70), only 7 patients (10%) died before day +100 posttransplantation. When those patients were excluded from the analysis, the difference in median time to RBC transfusion independence remained significant between the 2 groups (45 days vs 80 days; $P = .002$). Fourteen patients presented with additional causes of anemia (9 with severe bleeding, 3 with drug-induced acute hemolysis, and 2 with severe fungal infection), and were excluded from the analysis.

In the MUD group, there was no significant difference between patients with grade 0-I aGVHD and those with grade II-IV aGVHD (35 days vs 60 days; $P = .08$).

Our evaluation of ABO-major mismatch on erythroid and hematopoietic reconstitution in both PBSCT and CBT revealed no significant impact using these cell sources. These results are summarized in the Supplemental Material.

Correlation between ABO Incompatibility and GVHD Incidence

The probability of aGVHD was compared between the ABO-matched and ABO-mismatched transplants for the 3 different stem cell sources. A separate analysis was performed after exclusion of patients with bidirectional ABO incompatibility in subgroups in which significant difference has been observed (Table 4).

Among BM recipients, overall incidence of grade ≥2 aGVHD was similar in ABO-matched and ABO-mismatched transplants (72% [n = 154] and 76% [n = 172]; $P = .19$). No difference was observed between the MRD and MUD groups with ABO-mismatched transplants (data not shown).

We assessed the incidence of aGVHD in the group of PBSC recipients similarly. Analysis performed after exclusion of patients with major/minor mismatch (n = 77) showed a tendency toward higher aGVHD incidence in ABO-mismatched transplants (83%, vs 63% in ABO-matched; $P = .05$). This borderline statistically significant difference was observed in both transplants from MRDs (46% vs 62%; $P = .05$) and those from MUDs (68% vs 83%; $P = .04$). No difference in GVHD prophylaxis or conditioning regimen was observed in those 2 groups (Tables 1 and 2).

In CBT recipients, analysis of transplants from both the MRD and MUD groups did not appear to be feasible, because of the small number of patients.

Impact of ABO Major Incompatibility on Relapse Rate

The risk of relapse was evaluated after selection of patients undergoing HSCT for a malignant hematologic disease. Hematologic malignancies represented 74% (n = 292) of ABO-matched transplants and 71% (n = 296) of ABO-mismatched transplants (Tables 1 and 2).

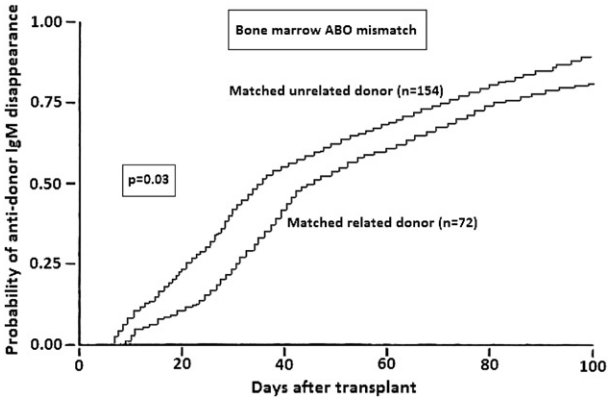

Figure 2. Probability of anti-donor IgM disappearance according to time after transplantation in ABO-mismatched transplants with MRDs and MUDs.

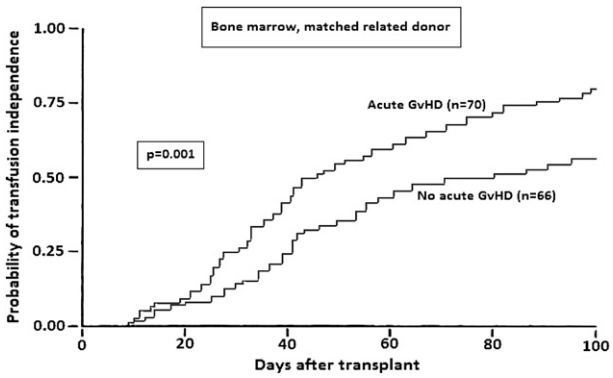

Figure 3. Probability of transfusion independence posttransplantation among ABO-mismatched BMT recipients (MRDs) according to GVHD occurrence.

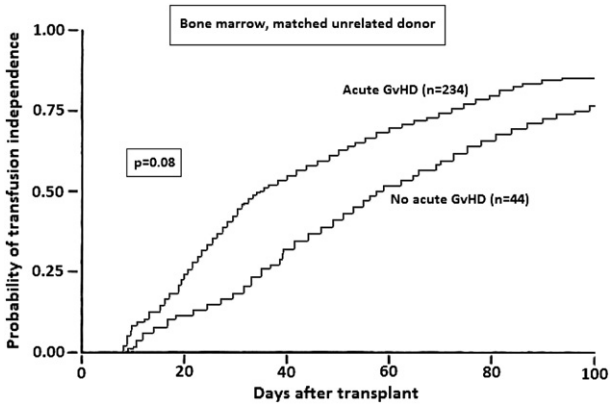

Figure 4. Probability of transfusion independence posttransplantation among ABO-mismatched BMT recipients (MUDs) according to GVHD occurrence.

Among BMT recipients, the relapse rate was 38% (n = 58) in ABO-matched transplants and 34% (n = 53) in ABO-mismatched transplants (P = .065). Analysis of the MRD and MUD groups for both ABO-matched and ABO-mismatched transplants demonstrated no significant difference in relapse risk (40% vs 43% in the MRD group [P = .11] and 33% vs 36% in the MUD group [P = .08]).

In both the PBSCT and CBT recipients, the relapse rate did not appear to be different in ABO-matched and ABO-mismatched transplants. Only a borderline statistical significance was observed in the PBSCT group, with a relapse rate of 41% (n = 45) in the ABO-matched group and 35% (n = 37) in the ABO-mismatched group (P = .055).

OS

OS was analyzed by comparing ABO-matched and ABO-mismatched transplants for BMT, PBSCT, and CBT recipients only in patients treated for hematologic malignancies. The median follow-up time for surviving patients was 76 months for MRDs (range, 26-245 months) and 68 months for MUDs (range, 21-228 months).

OS according to stem cell source is summarized in Table 5. In BMT recipients, there was no significant difference in overall survival at 5 years between the MRD group (51% vs 49%; P = .18) and the MUD group (38% vs 33%; P = .08). In PBSCT recipients, similar nonsignificant trends were found (MRD and MUD overall survival of 42 vs 46 months [P = .08] and 30 vs 38 months [P = .055] according to ABO match, respectively). Among CBT recipients, median overall survival was 20 months (n = 30) for ABO-matched transplants and 21 months (n = 35) for ABO-mismatched transplants (P = .06). Subgroup analysis in MRDs and MUDs could not be performed because of the small number of patients in this population.

Multivariate Analysis

We used Cox proportional hazard regression models to assess the impact of different transplantation parameters on 3-year OS, aGVHD and cGVHD incidence, and relapse rate. Among the parameters tested, we considered age, disease type, and status at time of transplantation, donor type, stem cell source, conditioning regimen, GVHD prophylaxis, ABO major mismatch, and hemagglutinin subtype. For OS and relapse risk, we considered grade ≥II aGVHD and cGVHD as time-dependent covariates. Moreover, relapse risk assessment in this multivariate analysis considered only patients with acute myelogenous or lymphoblastic leukemia, given the high heterogeneity of hematologic malignancies included in the study.

When considering 3-year OS, no impact of ABO major mismatch or hemagglutinin subtype on survival was identified (hazard ratio [HR], 1.07 [P = .45] and 0.97 [P = .35], respectively). Similarly, ABO mismatch did not significantly influence the aGVHD rate (odds ratio [OR], 1.24; P = .09). The sole parameter influenced by donor/recipient ABO mismatch was 3-year relapse risk (OR, 0.65; P = .04). This parameter also was influenced by cGVHD (OR, 0.55; P = .035). The results of multivariate analysis are summarized in Table 6.

DISCUSSION

The aim of this single-center retrospective study was to evaluate in a large cohort of patients the impact of donor/recipient major ABO mismatch on GVHD incidence, OS, and relapse risk according to stem cell

Table 4. Grade ≥II aGVHD Incidence in ABO-Matched and -Mismatched Transplants According to Stem Cell Source

Stem Cell Source	ABO Compatibility	n	Grade ≥II aGVHD, n (%)	Univariate RR	P Value	95% CI
BM	ABO match	213	154 (72)	1.0	NA	NA
	ABO mismatch	226	172 (76)	1.15	.19	1.08-1.28
PBSCs	ABO match	135	85 (63)	1.0	NA	NA
	ABO mismatch	138	113 (82)	2.10	**.01**	**1.8-3.1**
CB	ABO match	47	12 (26)	1.0	NA	NA
	ABO mismatch	50	19 (38)	1.21	.15	1.12-1.40

aGVHD indicates acute graft-versus-host disease; BM, bone marrow; PBSCs, peripheral blood stem cells; CB, cord blood.

Table 5. Median Overall Survival in ABO-Matched and ABO-Mismatched Transplants According to Stem Cell Source

Stem Cell Source	ABO Compatibility	Hematologic Malignancies, n (%)	Median Overall Survival, Months	P
BM	ABO match	153 (72)	38	NA
	ABO mismatch	156 (69)	41	.07
PBSCs	ABO match	109 (81)	34	NA
	ABO mismatch	105 (76)	39	**.055**
CB	ABO match	30 (64)	20	NA
	ABO mismatch	35 (70)	21	.35
All cohort	ABO match	292 (74)	34	NA
	ABO mismatch	296 (71)	37	.06

BM indicates bone marrow; PBSCs, peripheral blood stem cells; CB, cord blood.

source. All patients were treated in a single center and received homogeneous management in terms of conditioning regimen, indications for transplantation, GVHD prophylaxis, immunohematologic monitoring, and transfusion policies. Moreover, PBSCT and CBT recipients were included in the study, so that our series represents, to the best of our knowledge, one of the largest to date on the impact of major ABO mismatch in patients undergoing allogeneic HSCT.

To evaluate the relevance of our population selection, we first assessed the impact of ABO mismatch on erythroid reconstitution in BMT recipients. As described previously [8], we found a significantly longer time to transfusion independence in the ABO-mismatch population. The level of genetic disparity between donor and recipient also appeared to have an impact on both antidonor IgM hemagglutinin disappearance and RBC pack transfusion needs with faster disappearance of antidonor IgM hemagglutinins in MUD recipients and MRD recipients with grade ≥II aGVHD.

The median time to reach undetectable antidonor IgM tends to be shorter in MUD recipients with grade II-IV aGVHD compared with those with grade 0-I aGVHD, but the difference does not reach statistical significance, as reported previously [8]. The lack of correlation in this group was not related to a lack of power because of small sample size. We also investigated whether the higher mortality rate in MUD recipients with grade ≥II aGVHD could account for this lack of difference. The mortality rate before day +100 after MUD HSCT was 14% (n = 33) in patients with grade ≥II aGVHD, compared with 6% (n = 1) in those without grade ≥II aGVHD. Excluding these patients with early death did not change the results in term of transfusion needs or antidonor IgM hemagglutinin clearance.

Table 6. Multivariate Analysis of Factors Influencing Survival, aGVHD, and Relapse Risk

	Parameter	HR	95% CI	P
3-year overall survival	Age >35	2.56	1.95-3.35	.01
	No CR at transplantation	2.75	2.10-3.75	.005
	Grade ≥II aGVHD	2.25	1.85-3.05	.008
aGVHD incidence	Age >35	2.18	1.65-2.75	.02
	Unrelated donor	3.24	2.65-4.15	.001
	Peripheral blood	1.78	1.25-2.95	.03
Relapse risk	Donor/recipient ABO mismatch	0.65	0.58-0.72	.04
	cGVHD	0.55	0.43-0.72	.035

aGVHD indicates acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; CR, complete remission.

The hypothesis of a potential impact of early death rate in MRD patients with grade II-IV aGVHD on results has been verified. In 70 patients with grade II-IV aGVHD, only 10% (n = 7) died before day +100, compared with 6% (n = 4) of those with grade <II aGVHD. After excluding those patients with early death, the difference in median time to RBC transfusion independence remained significant between the 2 groups (45 days vs 80 days; $P = .002$).

When evaluating the impact of aGVHD on antidonor hemagglutinin titer clearance, we assessed the impact of conditioning regimen, particularly the use of ATG. ATG is known to target B cells and plasma cells through a complement-independent apoptosis mechanism [23,24]. ATG was given as GVHD prophylaxis in 102 of 154 patients receiving a MUD transplant with ABO-mismatched BM (66%). A similar median time to disappearance was found in patients who received ATG and those who did not receive ATG, suggesting that the role of an allogeneic effect against host plasma cells is independent of ATG use.

We also identified a direct correlation between antidonor IgM hemagglutinin clearance and RBC transfusion needs in this ABO-mismatched population receiving MRD transplants by excluding from the analysis all patients with confounding causes of severe anemia. Median time to RBC transfusion independence was confirmed to be shorter in MRD recipients with grade II-IV aGVHD, with this difference independent of all associated conditions. These results are consistent with those of previous studies [1,3,8,25] and give another argument to the hypothesis of a graft-versus-plasma cell effect in BMT recipients.

But, when considering erythroid reconstitution in PBSCT and CBT recipients, we found any impact of major ABO incompatibility, regardless of hemagglutinin type and number of nucleated cells in the transplant. The majority of PBSCT recipients received nonmyeloablative conditioning, and more than half of the patients (70/138) experienced prolonged erythroid reconstitution with this type of conditioning, in agreement with some [26-28], but not all [29], reports. The lack of impact of ABO mismatch in those transplants might result from the immunosuppressive treatment based on association of CsA with MMF,

because MMF has the ability to suppress B cell antibody production, and thus might possibly prevent pure red cell aplasia after low-dose conditioning [30]. Most of the CBT recipients (84%; 42/50) received standard conditioning with GVHD prophylaxis based mainly on CsA-MMF. The reasons for the absence of major ABO- mismatch impact on erythroid recovery in this population are unclear, but our results should be interpreted with caution because of the sample size–limited statistical power of the analysis. Thus, somewhat surprisingly, the graft-versus–plasma cell effect seems to be confined to BMT either directly or indirectly through aGVHD.

Our study revealed no impact of major ABO incompatibility on neutrophil or platelet recovery regardless of donor type, conditioning regimen, and stem cell source. These results are consistent with previous studies that reported no impact of major ABO mismatch on engraftment [27,31].

In particular, we identified 5 patients (4 BMT recipients and 1 PBSCT recipient) with pure RBC aplasia and very prolonged RBC transfusion needs (time to transfusion independence ranging from day +245 to day +545 posttransplantation), with one patient still dependent on packed RBC transfusion at time of follow-up (25 months). All patients underwent HSCT after removal of ABO-incompatible RBCs following current methods for erythrocyte depletion in donor/recipient major ABO-incompatible allogeneic HSCT [4,5]. Two patients became transfusion-independent after rituximab infusion, confirming the use of this anti-CD20 monoclonal antibody in this situation [32,33]. One patient recovered from pure RBC aplasia after plasmapheresis, and another did so after high-dose dexamethasone, as described previously [34,35]. The only patient with persistent RBC transfusion needs failed to respond to the aforementioned treatments followed by 3 doses of donor lymphocyte infusion, with no occurrence of aGVHD. She received 2 units of packed RBCs every other week, and had a reticulocyte count $<10\ 10^9$/L at day +750 posttransplantation.

In this study, ABO incompatibility did not significantly impair survival or aGVHD rates. As expected, cGVHD represented the major parameter associated with reduced relapse risk in multivariate analysis (HR, 0.55; $P = .035$). The association of ABO incompatibility and decreased relapse rate should be interpreted with extreme caution, however (HR, 0.65; $P = .04$). Note that this statistical significance has been identified only in patients with acute leukemia after exclusion of other malignancies. Indeed, comparison between acute leukemias, CML, MDS, and lymphomas does not appear to be feasible because of the high heterogeneity in relapse risk stratification at the time of transplantation. When focusing only on BMT recipients for relapse rate in patients with acute leukemia, no effect was found in ABO-mismatched transplants compared with ABO-matched transplants (36% vs 39%; $P = .12$). Moreover, during our relatively long study period, new risk factors have emerged (eg, flt3 ITD in AML and major role of cytogenetic classification in MDS and myeloma) that could not be included in these retrospective analyses.

Finally, we specifically explored correlation between cGVHD incidence and major ABO incompatibility, because little data are available on this subject. The only risk factor identified in multivariate analysis for cGVHD was PBSCT, with no effect of major ABO mismatch (HR, 1.04; $P = .35$; data not shown). This absence of correlation between chronic GVHD and major ABO mismatch might be linked to "donor-directed" instead of "host-directed" allogeneic activity in this situation, with cGVHD requiring sustained alloreactivity against minor histocompatibility antigens, which is more likely to occur in minor ABO-mismatched transplants.

In summary, in our experience, major ABO incompatibility leads mainly to delayed RBC recovery after BMT, but not after PBSCT or CBT. Major ABO mismatch does not seem to have a significant effect on other major outcomes after allogeneic HSCT, such as aGVHD and cGVHD incidence, relapse rate, and OS regardless of the stem cell source. Therefore, whenever feasible, major ABO-mismatched donors should be avoided in BMT recipients, to prevent delayed erythroid recovery with prolonged RBC transfusion needs and impaired quality of life.

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SUPPLEMENTARY DATA

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REFERENCES

1. Klumpp TR. Immunohematologic complications of bone marrow transplantation. *Bone Marrow Transplant*. 1991;8:159-170.

2. Petz LD. Hemolysis associated with transplantation. *Transfusion*. 1998;38:224-228.
3. Gmur JP, Burger J, Schaffner A, et al. Pure red cell aplasia of long duration complicating major ABO-incompatible bone marrow transplantation. *Blood*. 1990;75:290-295.
4. Braine HG, Sensenbrenner LL, Wright SK, et al. Bone marrow transplantation with major ABO blood group incompatibility using erythrocyte depletion of marrow prior to infusion. *Blood*. 1982;60:420-425.
5. Bensinger WI, Buckner CD, Clift RA, et al. Plasma exchange and plasma modification for the removal of anti-red cell antibodies prior to ABO-incompatible marrow transplant. *J Clin Apheresis*. 1987;3:174-177.
6. Hows J, Beddow K, Gordon-Smith E, et al. Donor-derived red blood cell antibodies and immune hemolysis after allogeneic bone marrow transplantation. *Blood*. 1986;67:177-181.
7. Gajewski JL, Petz LD, Calhoun L, et al. Hemolysis of transfused group O red blood cells in minor ABO-incompatible unrelated-donor bone marrow transplants in patients receiving cyclosporine without post-transplant methotrexate. *Blood*. 1992;79:3076-3085.
8. Mielcarek M, Leisenring W, Torok-Storb B, et al. Graft-versus-host disease and donor-directed hemagglutinin titers after ABO-mismatched related and unrelated marrow allografts: evidence of graft-versus-plasma cell effect. *Blood*. 2000;96:1150-1156.
9. Kimura F, Sato K, Kobayashi S, et al. Impact of ABO-blood group incompatibility on the outcome of recipients of bone marrow transplants from unrelated donors in the Japan Marrow Donor Program. *Haematologica*. 2008;93:1686-1693.
10. Bacigalupo A, Van Lint NT, Occhini D, et al. ABO compatibility and acute graft-versus-host disease following allogeneic bone marrow transplantation. *Transplantation*. 1988;45: 1091-1094.
11. Hows JM, Chipping PM, Palmer S, et al. Regeneration of peripheral blood cells following ABO incompatible allogeneic bone marrow transplantation for severe aplastic anemia. *Br J Haematol*. 1983;53:145-151.
12. Mehta J, Powles R, Sirohi B, et al. Does donor–recipient ABO incompatibility protect against relapse after allogeneic bone marrow transplantation in first remission acute myeloid leukemia? *Bone Marrow Transplant*. 2002;29:853-859.
13. Mielcarek M, Torok-Storb B, Storb R. ABO incompatibility and relapse risk in patients undergoing allogeneic marrow transplantation for acute myeloid leukemia [letter]. *Bone Marrow Transplant*. 2002;30:547-548.
14. Benjamin RJ, McGurk S, Ralston MS, et al. ABO incompatibility as an adverse risk factor for survival after allogeneic bone marrow transplantation. *Transfusion*. 1999;39:179-187.
15. Kalaycioglu M, Copelan E, Avalos B, et al. Survival after ABO-incompatible allogeneic bone marrow transplant after a preparative regimen of busulfan and cyclophosphamide. *Bone Marrow Transplant*. 1995;15:105-110.
16. Stussi G, Seebach L, Muntwyler J, et al. Graft-versus-host disease and survival after ABO-incompatible allogeneic bone marrow transplantation: a single-center experience. *Br J Haematol*. 2001;113:251-253.
17. Bensinger WI, Martin PJ, Storer B, et al. Transplantation of bone marrow as compared with peripheral-blood cells from HLA-identical relatives in patients with hematologic cancers. *N Engl J Med*. 2001;344:175-181.
18. Champlin RE, Schmitz N, Horowitz MM, et al. Blood stem cells compared with bone marrow as a source of hematopoietic cells for allogeneic transplantation. IBMTR Histocompatibility and Stem Cell Sources Working Committee and the European Group for Blood and Marrow Transplantation (EBMT). *Blood*. 2000;95:3996-4003.
19. Buckner CD, Clift RA, Sanders JE, et al. ABO-incompatible marrow transplants. *Transplantation*. 1978;26:233-238.
20. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc*. 1958;53:457-481.
21. Kalbfleisch JD, Prentice RL. *The Statistical Analysis of Failure Time Data*. New York: Wiley; 1980.
22. Cox DR. Regression models and life tables (with discussion). *J R Stat Soc B*. 1972;34:187-220.
23. Zand MS, Vo T, Pellegrin T, et al. Apoptosis and complement-mediated lysis of myeloma cells by polyclonal rabbit antithymocyte globulin. *Blood*. 2006;107:2895-2903.
24. Zand MS, Vo T, Huggins J, et al. Polyclonal rabbit antithymocyte globulin triggers B-cell and plasma cell apoptosis by multiple pathways. *Transplantation*. 2005;79:1507-1515.
25. Benjamin RJ, Connors JM, McGurk S, et al. Prolongederythroid aplasia after major ABO-mismatched transplantation for chronic myelogenous leukemia. *Biol Blood Marrow Transplant*. 1998;4: 151-156.
26. Maciej Zaucha M, Mielcarek M, Takatu A, et al. Engraftment of early erythroid progenitors is not delayed after non-myeloablative major ABO-incompatible haematopoietic stem cell transplantation. *Br J Haematol*. 2002;119:740-750.
27. Kim JG, Sohn SK, Kim DH, et al. Impact of ABO incompatibility on outcome after allogeneic peripheral blood stem cell transplantation. *Bone Marrow Transplant*. 2005;35:489-495.
28. Kanda Y, Tanosaki R, Nakai K, et al. Impact of stem cell source and conditioning regimen on erythrocyte recovery kinetics after allogeneic haematopoietic stem cell transplantation from an ABO-incompatible donor. *Br J Haematol*. 2002;118:128-131.
29. Bolan CD, Leitman SF, Griffith LM, et al. Delayed donor red cell chimerism and pure red cell aplasia following major ABO-incompatible non-myeloablative hematopoietic stem cell transplantation. *Blood*. 2001;98:1687-1694.
30. Veelken H, Wasch R, Behringer D, et al. Pure red cell aplasia after allogeneic stem cell transplantation with reduced conditioning. *Bone Marrow Transplant*. 2000;26:911-915.
31. Benjamin RJ, McGurk S, Ralston MS, et al. ABO-incompatible bone marrow transplantation: the transfusion of incompatible plasma may exacerbate regimen-related toxicity. *Transfusion*. 1999;39:1273-1274.
32. Maschan AA, Skorobogatova EV, Balashov DN, et al. Successful treatment of pure red cell aplasia with a single dose of rituximab in a child after major ABO incompatible peripheral blood allogeneic stem cell transplantation for acquired aplastic anemia. *Bone Marrow Transplant*. 2002;30:405-407.
33. Grzegorz H, Stella-Holowiecka B, Krawczyk-Kulis M, et al. Successful treatment of pure red cell aplasia with repeated, low dose of rituximab in 2 patients after ABO-incompatible allogeneic haematopoietic stem cell transplantation for acute myeloid leukaemia. *Haematologica*. 2005;90:e93-e96.
34. Rabitsch W, Knobl P, Prinz E, et al. Prolonged red cell aplasia after major ABO-incompatible allogeneic hematopoietic stem cell transplantation: removal of persisting isohemagglutinins with Ig-Therasorb® immunoadsorption. *Bone Marrow Transplant*. 2003;32:1015-1019.
35. Deotare UR, Vishwabandya A, Mathews V, et al. Response to high-dose dexamethasone for acquired pure red cell aplasia following ABO-mismatched allogeneic stem cell transplantation. *Bone Marrow Transplant*. 2006;37:1149-1150.